Atrial Natriuretic Peptide in Type 2 Diabetes Mellitus: Response to a Physiological Mixed Meal and Relationship to Renal Function

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Relatively few data exist on atrial natriuretic peptide (ANP) characteristics in Type 2 diabetes mellitus (DM). Therefore, plasma immunoreactive ANP concentrations were measured before and for 4 h following the ingestion of a physiological mixed meal in 8 newly diagnosed, normotensive, normoalbuminuric, patients with Type 2 DM and 6 normotensive, non-diabetic controls. In patients with Type 2 DM, basal plasma ANP concentrations were 4.0 ± 2.0 and not significantly changed following ingestion of the meal, with peak levels of 4.9 ± 2.8 pmol l^{-1}. Non-diabetic controls had higher basal plasma ANP concentrations, 8.7 ± 3.4 pmol l^{-1} ($p < 0.05$), significantly increasing to a peak of 11.9 ± 6.3 pmol l^{-1} at 30 min post meal. Extracellular fluid volume (ECV) was not different between diabetic patients and controls (15 877 ± 2679 vs 13 668 ± 1792 ml^3). Glomerular filtration rate (GFR) (isotopic clearance corrected for body surface area) was elevated in diabetic patients (mean ± SD) 130 ± 39 vs 98 ± 10 ml min^{-1}, $p < 0.05$). For the DM subjects, basal ANP levels were negatively correlated with GFR (r_s – 0.74, $p < 0.05$) and effective renal plasma flow (ERPF) (r_s – 0.8, $p < 0.05$). We conclude that patients with Type 2 DM demonstrate reduced basal plasma ANP concentrations which are inversely correlated to renal function. In contrast to non-diabetic controls, ANP in Type 2 DM does not rise in response to feeding. © **1998 John Wiley & Sons, Ltd.**

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Introduction

There is a close correlation between plasma atrial natriuretic peptide (ANP) and extracellular fluid volume.[1,2] Plasma ANP concentrations increase in response to volume expansion, hypertonic saline infusion[3], head-out body immersion,[4] and protein ingestion.[5] ANP provokes natriuresis, with reduced sodium reabsorption in the distal renal tubules.[1,6] In both Type 1 and Type 2 diabetes mellitus (DM), there is an average 10 % increase in total body sodium,[7] an increased incidence of hypertension[8] and hyperfiltration.[9] Abnormalities in ANP sensitivity or secretion may contribute to these features.

In well-controlled patients with Type 1 DM, basal plasma ANP concentrations have been reported as normal or elevated, with an absent or blunted response to volume expansion and water immersion.[10,11] In patients with raised plasma ANP concentrations, the kidney appears to have reduced responsiveness to exogenous ANP,[1,12,13] with reduced ANP binding in the renal cortex but not in the medulla.[13] The diuretic and natriuretic response to plasma ANP has been reported to be decreased in patients with Type 1 DM compared to normal controls, suggesting a down-regulation of ANP receptors or altered post-receptor signalling.[1] In streptozotocin-induced, insulinopaenic, diabetic rats, synthesis of ANP was augmented, reverting to normal with insulin therapy.[13]

In some studies higher plasma ANP levels have been reported in patients with either Type 2 or Type 1 DM and hypertension when compared with normotensive diabetic subjects.[12,14,15] These study results may have been biased by the inclusion of patients with established end-organ damage.

In patients with both types of DM, ANP concentrations rise with increasing urinary albumin excretion rates, especially with overt nephropathy,[10,12,16] increasing renal

Abbreviations: ANP atrial natriuretic peptide, ECV extracellular fluid volume, ERPF effective renal plasma flow, FPG fasting plasma glucose, GFR glomerular filtration rate, IRI immunoreactive insulin

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tubular enzyme excretion (both $\beta_2$microglobulin and N-acetyl glucosamine),[16] higher insulin concentrations,[15,17] and poor glycaemic control.[18] Plasma ANP concentrations have previously been demonstrated to rise with increasing renal dysfunction in patients with diabetes.[10,16] Many of these observations may be explained by impairment of the normal renal clearance of plasma ANP in nephropathy.

Studies in diabetic patients have concentrated predominantly on Type 1 DM. We therefore examined basal ANP concentrations and the response to a physiological mixed meal in patients with Type 2 DM, comparing them with normotensive non-diabetic controls.

Patients and Methods

Patients

We enrolled 8 normoalbuminuric (3 timed overnight urine collections with urinary albumin excretion rate < 5 μg min^{-1}) patients with Type 2 DM (5 male, median age (range), 52 (43–64) years). All the patients were studied within 1 week of presentation when they had received basic dietary advice but no other treatment. They were compared to 6 non-diabetic controls of similar age (3 male, median age, 58 (56–65) years) on no medication. Both the patients with DM and controls were normotensive, systolic pressure being (mean ± SD) 134 ± 21 vs 120 ± 16 and diastolic 84 ± 9 vs 75 ± 12 mmHg, respectively ($p > 0.005$). Compared to controls, those with diabetes had a higher body mass index (mean ± SD), 28.8 ± 4.7 vs 24.2 ± 3.1 kg m^{-2} ($p < 0.05$).

Sampling Methods

The study was carried out with local ethics committee approval and informed patient consent. Subjects were evaluated in the semi-recumbent position following an overnight fast. After lying for 30 min (designated minus 30 min) basal blood samples were obtained for immunoreactive insulin (IRI), fasting plasma glucose (FPG), haematocrit, urea, haemoglobin, and plasma ANP concentrations. Samples for IRI and ANP were collected into chilled sample bottles and stored on ice before centrifuging at 3500 rev min^{-1} and the serum frozen at –20°C until analysed.[19] Blood sampling was repeated at time 0, after which subjects consumed a standardized 560 kcal physiological mixed meal (50.2 % carbohydrate, 36.8 % fat, 12.9 % protein) within 10 min. Thereafter samples for PG, IRI, and ANP were collected every 30 min for 4 h. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured on the subsequent day, using the technique of a single i.v. injection of ^{51}Cr-EDTA and ^{125}Iodohippurate for the simultaneous determination of GFR and ERPF, as previously described by Vora *et al.*[9]

Analytical Methods

Plasma glucose was measured by the glucose oxidase technique using an autoanalyser. IRI was assayed by a double antibody technique using reagents from NOVO biolabs, Bagsvaerd, Denmark.[20] ANP immunoreactivity in plasma was measured by a specific radioimmunoassay which detects 1-alpha human ANP.[4,21] GFR and ERPF were estimated by gamma counting and standard formulae. Results were corrected to a body surface area of 1.73 m^2. Extracellular fluid volume (ECV) was calculated by the modified Brochner-Mortensen method.[22–24]

Statistical Analysis

All data are quoted as mean ± SD unless otherwise stated. The plasma ANP response to the meal, time 0 vs time in 30-min intervals from the meal, was analysed in each group using the Wilcoxon two-sample test. The differences between plasma ANP concentrations throughout the study in the patients with NIDDM and the controls were analysed using the Mann-Whitney U-test. Two-way ANOVA was used to test the differences in ECV and peak plasma ANP concentrations in the two groups. Spearman Rank correlation analysis was used to examine the relationship between basal (mean of measurements at time –30 and 0 min) plasma ANP concentrations vs BMI, systolic and diastolic blood pressure, HbA_1, FBC, haematocrit, urea, IRI, FPG, GFR, and ERPF. The correlation was also analysed after log transformation of the GFR values.

Results

The patients with Type 2 DM had significantly higher HbA_1, 10.9 ± 3.2 vs 7.4 ± 0.5 % ($p < 0.01$), fasting plasma glucose 10.5 ± 3.0 vs 5.8 ± 0.3 mmol l^{-1} ($p < 0.05$), and immunoreactive insulin concentrations 24.7 ± 23.1 vs 22.4 ± 5.8 mu l^{-1} ($p < 0.05$). As expected the glucose and IRI concentrations increased following the mixed meal. There was no significant difference in blood pressure for the 4 h of the study in either group. ECV was similar for patients with Type 2 DM and controls, median (95 % CI), 16 110 (13 800–18 220) vs 14 160 [11 790–15 550] ml^3, respectively (Mann-Whitney, $p > 0.05$; two-way ANOVA, $p > 0.05$). Plasma urea and haematocrit were non-significantly higher in the Type 2 DM group.

Plasma ANP concentrations were lower in patients with Type 2 DM than in the control group throughout

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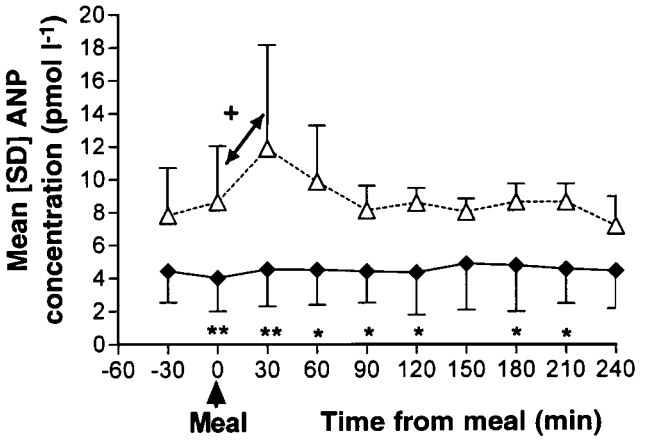

Figure 1. Response of mean plasma ANP (pmol l^{-1}) (mean (SD)) following a mixed meal in subjects with Type 2 DM (--◆--) and non-diabetic controls (--Δ--). Mann-Whitney U-test difference between controls and patients with Type 2 DM: *$p < 0.05$ and **$p < 0.01$. In controls, increase in plasma ANP between 0 and 30 min from meal, Wilcoxon 2-sample test: + $p < 0.05$. No significant difference in plasma ANP concentration in patients with Type 2 DM in response to meal

the majority of the study period ($p < 0.05$) (Figure 1). In the patients with DM the basal plasma ANP concentration was 4.0 ± 2.0 pmol l^{-1}, significantly reduced compared to the controls, 8.7 ± 3.4 pmol l^{-1} ($p < 0.01$). There was failure of the mixed meal to elicit a significant increase in the plasma ANP concentration in those with diabetes. The peak mean plasma ANP concentration was 4.9 ± 2.8 pmol l^{-1} 150 min after the meal in the patients with diabetes ($p > 0.05$ vs basal). By contrast, in the controls mean plasma ANP concentrations rose to a peak of 11.9 ± 6.3 pmol l^{-1} ($p < 0.01$ vs basal), at 30 min following the meal. The peak plasma ANP concentration in the diabetic group vs controls was confirmed as being significantly lower (Mann-Whitney, $p < 0.005$; two-way ANOVA, $p < 0.0001$).

GFR was elevated for the patients with Type 2 DM, 130 ± 39.3 vs 98.3 ± 9.8 ml min^{-1} in the control group ($p < 0.05$). ERPF was not significantly different, 541 ± 149 vs 430 ± 41 ml min^{-1} ($p > 0.05$). The patients with Type 2 DM had a significant negative correlation between basal plasma ANP concentrations and GFR ($r_s = -0.74$, $p < 0.05$) and also between basal plasma ANP and ERPF ($r_s = -0.80$, $p < 0.05$). This relationship was confirmed following logarithmic transformation of the GFR results ($r_s = -0.93$, $p < 0.005$). There was no other significant correlation between basal ANP and any of the other parameters in either group, notably the metabolic parameters.

Discussion

This study in patients with Type 2 DM demonstrates decreased plasma ANP concentrations with no response to a mixed meal when compared to non-diabetic subjects of a similar age. Suppression of plasma ANP concentrations has previously been reported in severely hyperglycaemic mice[25] and in Type 2 DM patients complaining of thirst.[26] In these studies the mice and DM patients had raised urea, haematocrit, sodium, and osmolality when compared to normoglycaemic mice or well-hydrated DM subjects.[25,26] In the current study there were no symptoms or biochemical evidence of dehydration in our patients with Type 2 DM and indeed the ECV concentrations were not significantly different compared to the control group.

Previous studies that have reported raised plasma ANP concentrations involved more heterogeneous populations than ours,[14,18] patients recruited having either Type 2 or Type 1 DM, with or without hypertension, varying degrees of renal dysfunction, and with concomitant use of various medication.[27,28] We chose to study newly diagnosed patients with Type 2 DM with no complications of diabetes, who were normotensive, normoalbuminuric, and on no current medication. By selecting this group of patients some of the confounding variables of other studies have been avoided.

The standard meal produced a normal physiological increase in plasma ANP concentrations in the control group but failed to elicit an increase in the subjects with DM. The hyperglycaemia and/or hyperinsulinaemia in the Type 2 DM population may have acted via an unknown mechanism to inhibit the release of ANP in response to the meal. Previous research has left it uncertain as to whether ANP plays an important role in baseline conditions with normal extracellular fluid volume. Plasma ANP appears to be an important factor in causing natriuresis induced by acute volume expansion. In addition pharmacological plasma concentrations of ANP can reduce blood pressure, suppressing the renin–angiotensin–aldosterone system (RAS).[6,19,29] We may speculate that the excess total body sodium, characteristic of diabetes mellitus prior to the development of hypertension or renal disease, in the absence of a high sodium intake, may in part be secondary to low plasma ANP concentrations and may possibly contribute to the development of hypertension.

ANP has been suggested as a factor involved in the evolution of the hyperfiltration,[2,6,30–32] characteristically associated with early diabetes mellitus.[9] A major determinant of GFR is the capillary hydraulic pressure (Pgc). It has previously been demonstrated that infusion of ANP produces an increase in Pgc and thus GFR, associated with a decrease in afferent arteriolar resistance and an increase in efferent arteriolar resistance.[1] By contrast, various studies also report that infusion of ANP antiserum or ANP receptor antagonists into streptozotocin-induced diabetic rats decreased GFR.[2,30–32]

There is redistribution of blood flow from the renal cortex to the medulla with an increase in vasa recta blood flow, leading to dissipation of the medullary solute gradient and reduced reabsorption of fluid from the medullary collecting duct.[1] It has been postulated that ANP produces natriuresis as a result of increased GFR

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with reduced sodium reabsorption in the distal renal tubules. Not all previous studies confirm the increase in GFR with raised ANP concentrations; at low doses ANP causes a natriuresis without any detectable change in GFR.[30] GFR in our population of patients with newly diagnosed Type 2 DM correlated negatively with basal ANP concentration, suggesting that ANP does not have a significant role in the evolution of hyperfiltration in recent onset Type 2 DM. The relationship may reflect increased ANP clearance and thus lower blood concentrations with increasing GFR.[10,16]

We conclude that in patients with newly diagnosed Type 2 DM and normal extracellular volume, basal plasma ANP concentrations are lower than those in control subjects and fail to increase in response to the stimulus of a physiologically mixed meal, in contrast to the situation in controls. In those with Type 2 DM, the negative correlation between basal plasma ANP concentrations and GFR implies that ANP is not involved in the increased GFR in these patients.

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